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Research Article

Physicochemical, microbial, and sensory properties of 'in-package' fermented *lassi*

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Abstract

This study investigated the physico-chemical characteristics, microbiological properties and sensory evaluation of starter culture-added "in-package" *lassi* (SC-IPL), compared to a control sample of *lassi* prepared using conventional approach. The results showed consistent titratable acidity across both samples, with pH values slightly higher in SC-IPL compared to the control. The total solids content was higher in the control than in SC-IPL, while moisture content was higher in SC-IPL (78.27±0.09%). SC-IPL had significantly lower fat content and higher protein levels than the control. Ash content, lightness value, and b* value were also lower in SC-IPL, although the samples were not significantly different in terms of a* value. The free fatty acid and tyrosine levels were higher in SC-IPL, while "wheying off" was also more prominent in SC-IPL. Both samples exhibited shear-thinning flow behaviour with SC-IPL showing a lower consistency index. Microbiologically, the control had higher SPC and LAB counts, with no coliforms, yeast, or molds detected in either sample. Sensory analysis revealed that SC-IPL scored higher in flavour, consistency, acidity, and overall acceptability (89.00±3.55), making it more favourable than the control sample.

Keywords: Buttermilk, Fermentation, In-package, *Lassi*

Highlights

- *Lassi* produced in-package method exhibited higher protein content and better sensory scores than the control.
- In-package minimized post-processing contamination thus reducing microbial load in the final product.
- The developed *lassi* had favourable physicochemical, microbiological, and sensory properties.
- Cost-effective and hygienic production method suitable for commercial dairy manufacturing.

INTRODUCTION

Lassi stands as a widely popular, ready-to-serve fermented milk beverage. It is prepared by adding a starter culture into heat-treated milk and subsequently diluting it with sugar. This delightful drink possesses a sweet fragrance, a slightly tangy taste, and a rich creamy texture. *Lassi* isn't merely a tasty beverage; it also offers therapeutic qualities, delivering a revitalizing experience (George *et al.*, 2010). In India, commercial cultured buttermilk or *lassi* is produced by adding starter culture to low-fat milk, followed by fermentation, homogenization, and dilution with water (Mudgil *et al.*, 2016). Assessing the sensory attributes of milk and its derivatives relies on three primary factors: colour and appearance, flavour and texture. To prevent the development of undesirable microbial flavours, maintaining proper sanitation is essential. Thoroughly cleaning all equipment surfaces before use and sanitizing milk-related machinery are critical steps. Neglecting this can result in milk contamination, leading to the emergence of objectionable flavours. Notably, in dairy processing plants, post-pasteurization contamination stands as a significant contributor to spoilage (Molina *et al.*, 2009).

Post processing contamination is a significant challenge for the dairy industry, limiting the shelf life of milk. Bacterial spoilage can occur when bacteria enter the product after heat treatment, often during filling and packaging, and can survive refrigeration temperatures. Simply inspecting the final products is frequently insufficient to ensure quality, as no further adjustments can be made once the product is packaged. As a result, industries are shifting their focus towards controlling the production process rather than solely evaluating the end products (Belloque *et al.*, 2009).

Traditional food processing methods are essential in creating conventional diets. However, with the increasing demand for nutritious, safe, and affordable food products, industries are continually seeking improved processing techniques. Over time,

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advancements in technology, the development of modern, sophisticated equipment, and extensive scientific research have led to innovative approaches to food fermentation. Keeping in mind, this technology was developed by fermenting the *lassi* within the packaging material, and its chemical, sensorial and microbiological properties were studied.

MATERIALS AND METHODS

For the preparation of the starter culture, added "in-package" *lassi* (SC-IPL), raw milk procured from the Experimental Dairy Unit of NDRI, Karnal was standardized to 1% fat and 7% SNF, followed by the addition of WPC-80, 0.2% CMC and 13% sugar syrup (w/w basis of milk). The milk then underwent a two-stage homogenization process, followed by heat treatment at 90°C for 20 minutes, followed by cooling to 42°C. Following these steps, the milk was inoculated with the NCDC 263 starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* sub sp. *bulgaricus*). The prepared milk was subsequently transferred into glass bottles and allowed to incubate until the acidity reached 0.70% lactic acid. To convert the resulting curd into *lassi*, the packaged curd was cooled to 25°C and agitated in an orbital shaker incubator set at 350 rpm for 30 seconds. The final product was then subjected to physicochemical, sensorial and microbiological analysis.

Titratable acidity and moisture content in all samples were analyzed following the AOAC (2019) method. The pH of each sample was measured using a pH meter. Total solids were assessed in accordance with IS: SP (PART XI), 1981. Wheying off was determined using a modified centrifugation method based on Keogh and O'Kennedy's (1998) procedure. Total free fatty acid content in the developed product was estimated with slight modifications to the Deeth and Fitz Gerald (1976) method. The Hunter Lab colour system, along with Universal Software V 4.10, was employed to record L*, a*, and b* values. Sensory scores from seven semi-trained panel members were recorded using a composite scoring technique, evaluating flavour, consistency, acidity, colour, and appearance. To assess the flow behaviour of the samples, a rheometer equipped with a CP75 probe and Rheoplus software version 3.61 was utilized. The standard plate count, lactic acid bacteria count, coliform count, and yeast and mold count for the developed product were determined as per the method established by Houghtby *et al.* (1992).

RESULTS

Physicochemical characteristics: The physicochemical characteristics of SC-IPL and control *lassi* are detailed in Table 1. The titratable acidity (TA) values were 0.71±0.01% for the control and 0.70±0.01% for SC-IPL, with no significant differences. The pH values were 4.39±0.01 and 4.42±0.01 for control and SC-IPL, respectively, indicating a slightly higher pH in SC-IPL. All the pH values are significantly ($p<0.05$) different from each other. Total solids (TS) content was significantly higher in the control (24.29±0.09%) compared to SC-IPL (21.73±0.09%). Moisture content was significantly higher in SC-IPL (78.27±0.09%) than in the control (75.71±0.18%). Ash content represents the mineral and non-organic material present in the substance. SC-IPL had significantly ($p<0.05$) lower ash content, i.e., 0.46±0.01%, than the control sample (0.57±0.06%).

The fat content in SC-IPL was lower (0.76±0.01%) compared to the control (2.20±0.04%). However, SC-IPL had higher protein content (4.28±0.04%) than the control (3.70±0.04%). Ash content was slightly lower in SC-IPL (0.46±0.01%) compared to the control (0.57±0.06%).

Total free fatty acids (FFA) encompass the cumulative content of all free fatty acids in a sample. While milk typically contains minimal levels of FFA, it can have a noticeable impact on the flavour and aroma of fermented dairy products (Amores and Virto, 2019). In this analysis,

Table 1. Physicochemical characteristics of SC-IPL vis-vis control

Parameters	Control *lassi*	SC-IPL
Total solids (%)	24.29^{a}±0.18	21.73^{b}±0.09
Moisture content (%)	75.71^{b}±0.18	78.27^{a}±0.09
Fat (%)	2.20^{a}±0.04	0.76^{b}±0.01
Protein (%)	3.70^{b}±0.04	4.28^{a}±0.04
Ash (%)	0.57^{a}±0.06	0.46^{b}±0.01
L*	87.21^{a}±0.04	86.17^{b}±0.12
a*	-2.34^{a}±0.04	-2.42^{a}±0.03
b*	9.92^{a}±0.07	9.12^{b}±0.06
Titratable acidity (%)	0.71^{a}±0.01	0.70^{a}±0.01
pH	4.39^{a}±0.01	4.42^{a}±0.01
Total FFA (µg/mL sample)	0.32^{b}±0.01	0.45^{a}±0.02
Tyrosine (µg/mL sample)	10.40^{a}±0.16	10.59^{a}±0.08
Wheying off (mL/10mL sample)	0.75^{b}±0.10	2.18^{a}±0.06

Data represented as Mean ± SD; (n=3); ^{a,b} means with different superscripts in a row differ significantly ($p<0.05$) from each other the total free fatty acids were found to be significantly ($p<0.05$) higher for SC-IPL (0.45±0.02 µg/mL) as compared to the control (0.32±0.01 µg/mL). Tyrosine, an amino acid found in milk and dairy products, exhibited the highest content in SC-IPL at 10.59±0.08 µg/mL per sample, compared to the control at 10.40±0.16 µg/mL per sample. Wheying off refers to the phenomena of separating whey from curd in dairy products like *lassi, dahi*, and yoghurt. This occurs when the protein-gel matrix is disrupted, causing whey to seep out of the matrix. Significantly ($p<0.05$) higher wheying off was observed in SC-IPL (2.18±0.06a mL/10mL sample) compared to the control (0.75±0.10 mL/10mL sample).

The flow behaviour of both control and SC-IPL was recorded (Table 2). For both control and SC-IPL, the power law model was found to be the most suitable fit. The control sample exhibited a higher consistency index. Both the samples exhibited a shear-thinning nature with a flow behaviour index value of $n<1$.

Colour analysis: Hunter Lab colour values revealed that the lightness value (L*) was higher for the control (87.21±0.04) compared to SC-IPL (86.17±0.12). No significant differences were observed in the redness-greenness spectrum (a* value), but the yellowness-blueness spectrum (b* value) was significantly lower in SC-IPL (9.12±0.06) compared to the control (9.92±0.07).

Microbiological analysis: The microbial analysis showed higher standard plate counts (SPC) in the control (10.50±0.02 log cfu/mL) compared to SC-IPL (10.33±0.01 log cfu/mL). Similarly, lactic acid bacteria (LAB) counts were higher in the control (10.36±0.01 log cfu/mL) than in SC-IPL (10.21±0.02 log cfu/mL). No coliforms, yeast, or molds were detected in any sample as detailed in Table 3.

Table 2. Flow behaviour of SC-IPL and control *lassi*

Model	Parameters	Control	SC-IPL
Newtonian model	R^2	0.813	0.474
	η	0.100	0.034
Bingham plastic model	R^2	0.563	0.897
	η	0.070	0.025
	$\sigma 0$	2.008	0.602
Power law model	R^2	0.989	0.961
	K	1.916	0.408
	n	0.303	0.410
Herschel bulkley model	R^2	0.900	0.946
	K	0.399	0.044
	n	0.601	0.880
	$\sigma 0$	2.008	0.602

R^2 is regression coefficient; K is consistency index; n is flow behaviour index; η is viscosity; $\sigma 0$ is yield stress

Table 3. Microbiological analysis of the SC-IPL and control *lassi*

Parameter	Control	SC-IPL
SPC count (log cfu/mL)	10.50^a±0.02	10.33^b±0.01
LAB count (log cfu/mL)	10.36^a±0.01	10.21^b±0.02
Coliform count (log cfu/mL)	ND	ND
Yeast and mold count (log cfu/mL)	ND	ND

Data represented as Mean ± SD, (n=3); ^{a,b} means with different superscripts in a row differ significantly ($p<0.05$) from each other; ND: Not detected

Sensory attributes: Sensory evaluation revealed higher scores for SC-IPL in flavour (41.43±2.70), consistency (31.21±1.47), acidity (7.93±0.77), and overall acceptability (89.00±3.55), compared to the control, as presented in Table 4.

DISCUSSION

Physicochemical characteristics: The titratable acidity values for SC-IPL and control samples were consistent, aligning with the findings of Gebreselassie *et al.* (2016), where similar acidity levels were observed in naturally cultured buttermilk. The slightly higher pH in SC-IPL indicates reduced lactic acid production, potentially due to the in-package fermentation process, which limits oxygen exposure and promotes controlled fermentation.

The lower total solids content in SC-IPL can be attributed to its reduced fat and solid-not-fat (SNF) levels, as the control was prepared using milk with higher initial fat content. This was consistent with previous reports on reduced-fat dairy products (Roy, 2023). Moisture content was higher in SC-IPL, contributing to its softer texture. The inclusion

Table 4. Sensory attributes of SC-IPL and control *lassi*

Sample	Flavour	Consistency	Acidity	Colour and appearance	Overall acceptability
Control	40.00^a±1.29	31.00^a±1.29	7.57^a±0.73	8.71^a±0.95	87.29^a±2.51
SC-IPL	41.43^a±2.70	31.21^a±1.47	7.93^a±0.77	8.43^a±0.79	89.00^a±3.55

Data represented as Mean ± SD, (n=3); No significant difference ($p>0.05$)

of whey protein concentrate (WPC-80) in SC-IPL resulted in higher protein content, as similarly reported by Kumar *et al.* (2020) in herbal *lassi*.

Khurana (2006) studied low-calorie fruit *lassi* and found the ash content ranged between 0.55-0.65% for different variants of fruit *lassi*. These findings are comparable to the results observed in this study, highlighting variability in ash content depending on the type and formulation of *lassi*.

Sharma *et al.* (2016) developed synbiotic *lassi* containing honey and observed that the free fatty acid content in both the control and synbiotic *lassi* samples was 0.56±0.03 and 0.60±0.02 μg/g, respectively, which aligns with our findings of increased FFA in the SC-IPL sample.

Similarly, Maurya *et al.* (2020) reported that the initial tyrosine content of both the control and experimental samples ranged between 10-15 μg/0.5 mL, supporting the observed tyrosine levels in this study. Factors such as milk type and processing methods likely influenced the variations in tyrosine content.

Penna *et al.* (2001) conducted a study on lactic acid beverages and found that the power law model provided the best fit, with determination coefficients (R^2) ranging between 0.898 and 1.000. This aligns with the findings in this study, confirming the suitability of the power law model for analyzing the flow behaviour of such products.

Colour analysis: The lightness value (L*) was slightly lower in SC-IPL, which may be due to the reduced fat content that influences the scattering of light. The b* value, representing yellowness, was also lower in SC-IPL, likely affected by the addition of WPC-80, which alters the visual appearance. These results align with studies by Dudhate *et al.* (2024), where similar variations in colour indices were observed in *lassi* with added fruit pulp.

Microbiological analysis: The lower microbial load in SC-IPL highlights the benefits of in-package fermentation in minimizing post-processing contamination. The reduced SPC and LAB counts compared to the control may result from the controlled fermentation environment, limiting microbial proliferation. The absence of coliforms, yeast, and molds in both samples suggests effective processing and packaging practices, aligning with previous studies emphasizing hygienic production methods (Khunte *et al.*, 2022).

Sensory attributes: Higher sensory scores for SC-IPL, particularly in flavour and consistency, underscore the enhanced consumer acceptability of the product. The use of in-package fermentation likely preserved the flavour and texture better by preventing oxidation and contamination during processing. Similar findings were reported by Sharma *et al.* (2016), where enhanced sensory properties were observed in honey-based symbiotic *lassi*. The overall acceptability of SC-IPL suggests it can serve as a cost-effective, consumer-preferred alternative to conventional *lassi*.

Starter culture added "in-package" *lassi* (SC-IPL), which was successfully developed and had comparable physicochemical and microbiological properties to that of *lassi* prepared using the conventional method. The flow behaviour of both SC-IPL and control *lassi* was best explained by power law model. In terms of sensory, the developed "in-package" *lassi* was more preferred even after having lower fat and SNF content which concludes that the developed product will be cost-effective and beneficial for dairy product manufacturers. Also, the low microbial load and "in-package" fermentation will allow manufacturers to prevent post-processing contamination in the final product.

Conflict of interest: The authors have declared that no conflict of interest exists in the study.

Author's contribution: P: Conducting the experiments and writing the draft; KK: Lead of the project, conception and design of the article and interpretation of the findings; WP: Data analyses, revision of the manuscript.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

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